IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:
Irene Quenville et al.	: Confirmation No.: 2124
	:
Serial No. : 10/725,233	: Examiner: Gregory R. Delcotto
Filed: December 1, 2003	: Art Unit: 1796
	:
Title: STABILITY ENHANCEMENT OF SOLUTIONS CONTAINING ANTIMICROBIAL AGENTS	: Attorney Docket No.: P03346
	:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF BRIEN DAVID UNDER 37 C.F.R. § 1.132

I, Brien C. David declare as follows:

1. I am presently employed as a Research Scientist at Bausch & Lomb ("B&L"), and I have been employed at B&L since October 2005. One of my primary responsibilities during this time, and to this day, includes the supervision and management of the microbiology laboratory, which supports the development of lens care solutions and other ophthalmic formulations. I graduated from the State University of New York at Plattsburgh in 1979 with a B.S. in microbiology and biology.

2. Attached to this Declaration is a redacted version of a Record of Invention (ROI) submitted to the Patent Legal Department by Irene Quenville, a co-inventor of the subject matter claimed. The statements made in the Record of Invention are incorporated into this Declaration with the following clarifications: Exhibits 1 and 2 correspond to Figures 1 and 3 of the application, respectively. The ROI provides an excellent backdrop as to why B&L investigated the use of a poly(ethylene terephalate) (PET) container to store a lens care solution that contains PHMB and a surfactant.

3. The biocidal reduction data that is discussed and attached to the Declaration was obtained in the microbiology laboratory at B&L. The biocidal data includes the raw biocidal log reduction data that was used in-part to prepare the graphical representations depicted in Figures 1 and 3 of the application at issue. I have also reviewed Tables 1 thru 8, which were prepared by Joseph Barrera, a B&L patent attorney, from the biocidal log reduction data obtained with a multipurpose lens care solution of test solution 1 listed on

page 14 of the application. The data listed in Tables 1 thru 8 is the raw biocidal log reduction data and the calculated 4-hour mean values for the separate trial runs. Five trials were used to determine the 4-hour mean value for test solution 1 in a PET container. Three trials were used to determine the 4-hour mean value for test solution 1 in a HDPE container.

4. Initially, I would like to explain how one of ordinary skill in the art of contact lens care development would interpret biocidal log reduction data as presented in this Declaration. First, although the data is reported as a log reduction at 1-hour and 4-hour it is the 4-hour data that is of greater technical significance and of greater technical accuracy. Also, the 4-hour data represents the labeled soak time for a multipurpose lens care solution, and more importantly, is the ISO (FDA) pass/fail time point for test development of such solutions. Second, the data is reported as a log reduction kill, that is, a one log reduction accounts for the killing of 90% of the microbes added to a disinfecting solution. For example, if one was to add 10,000 live microbes to a 10 mL solution containing a disinfectant and wait four hours, a one log reduction would mean that 9000 microbes or 90% of the microbes were killed and a three log reduction would mean that 9990 microbes or 99.9% of the microbes were killed by the disinfectant solution.

5. The ISO (FDA) pass/fail threshold for four hour disinfection is a 3.0 log kill for the three bacterium; *Staphylococcus aureus (Sa), Pseudomonas aeruginosa (Pa)*, and *Serratia marcescens (Sm)* and a 1.0 log kill for the two fungi; *Candida albicans (Ca)* and *Fusarium solani (Fs).*

6. Each of the reported log reduction values has an error of 0.5 log. Much of the error associated with each value originates from the repetitive dilutions of the disinfecting solutions following the inoculation of the solutions with the microbes and the very large numbers of microbes (about 500,000) one adds to 10 mL of disinfecting solution. Also, there exists some variance in the resistance of one microbe preparation to another on a given day in spite of efforts to maintain identical growth environments in which the microbes are grown both before and after inoculation of a solution. To account for this error of measurement, one of ordinary skill looks for trends in the data and not necessarily to specific log reduction values.

6. Microbes are grown on agar (a growth medium) in a controlled environment. A swab of microbes is transferred to a buffered saline solution and a number of repetitive dilutions are made to provide a stock solution of about 500,000 CFU/mL. The concentration of microbes in the stock solution is confirmed using UV-visible spectrophotometry (optical density measurements). One mL of this stock solution is added to 10 mL of disinfecting solution. Following a one or four hour exposure of the solution to the microbes, a 1 mL sample is removed and additional dilutions are made with buffered saline. Eventually, a diluted sample is placed on an agar plate and any live microbes remaining in the sample are permitted to replicate during a 48 hour incubation period, i.e., each living microbe forms a visible colony, which are then counted. The

number of observed colonies is then used to determine the log reduction value based on the number of dilutions made in the sample preparation.

7. I have reviewed Tables 1 thru 8 attached to this Declaration. Tables 1 and 4 include the raw and mean initial time data for test solution 1 in PET and high density polyethylene (HDPE), respectively. The components of test solution 1 are listed on page 14 of the application and includes 1.2 ppm of PHMB and 3 wt.% of nonionic surfactant.

8. Test solution 1 was packaged and stored in PET and HDPE containers for three months at 40 °C and for six months at 40 °C. If one of ordinary skill compares the mean initial data in each of Tables 1 and 4 with the mean biocidal data reported in Tables 7 and 8 for each of PET and HDPE, one would conclude that test solution 1 remains biocidal active in the PET container but not in the HDPE container. Particular attention is directed to the 4-hour mean data related to *Staphylococcus aureus (Sa), Candida albicans (Ca)* and *Fusarium solani (Fs),* which is summarized in Tables 7 and 8. The dramatic difference in biocidal stability of test solution 1 packaged and stored in PET verses the exact same solution in HDPE is biologically significant and is not to be expected. Conclusions derived from the 4-hour mean biocidal data are as follows.

a. There is statistically no change in the biocidal efficacy of test solution 1 in the PET container with respect to all five microorganisms at three months.

In contrast, there is 10-fold reduction in biocidal efficacy against *Pseudomonas aeruginosa* and greater than a 100-fold reduction against *Fusarium solani* of test solution 1 in HDPE.

b. There is no significant loss in biocidal activity of test solution 1 in the PET container with respect to four of the five microorganisms. The exception is a 10-fold reduction against *Fusarium solani*, i.e., a log reduction from 3.5 to 2.4. More importantly, test solution 1 stored in a PET container for 6 months at 40 °C is able to pass the ISO(FDA) standard for all five microorganisms.

In contrast, test solution 1 in the HDPE container exhibits a 100-fold reduction against *Pseudomonas aeruginosa*, greater than a 10-fold reduction against *Candida albicans* and is virtually inactive against *Fusarium solani*. In fact, test solution 1 stored in HDPE would fail the ISO(FDA) standard for two of the five microorganisms.

All statements made by the declarant, which are based on personal knowledge, are true or believed to be true. Furthermore, these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment or both and can jeopardize the validity of any US patent that may issue from the above-identified application.

Brien David

Date: 10/14/08

Table 1. Log Reduction of PHMB Formulation in PET; Initial Time

Trial No.	t (hr)	Sa	Pa	Sm	Ca	Fs
1	1	3.6	4.5	2.5	2.6	1.8
	4	4.0	4.5	4.6	3.5	3.0
2	1	4.4	4.6	3.9	2.5	2.3
	4	4.9	4.6	4.7	3.9	3.8
3	1	4.6	3.4	2.6	2.9	3.7
	4	4.6	4.7	4.6	3.7	4.2
4	1	3.4	4.2	3.0	3.0	2.8
	4	4.8	4.6	4.6	3.8	3.9
5	1	4.8	2.4	2.8	1.5	1.7
	4	4.9	4.1	3.0	3.0	2.7
mean	**4**	**4.6**	**4.5**	**4.3**	**3.6**	**3.5**

Table 2. Log Reduction of PHMB Formulation in PET; 3 Months, 40 °C

Trial No.	t (hr)	Sa	Pa	Sm	Ca	Fs
1	1	4.6	3.7	1.2	3.0	1.9
	4	4.9	4.9	3.2	4.0	3.1
2	1	2.7	2.8	3.1	3.0	2.3
	4	4.6	4.6	4.6	3.6	3.2
3	1	4.8	4.6	4.6	3.4	2.7
	4	4.8	4.6	4.6	4.4	3.9
4	1	2.5	4.7	4.0	2.9	1.9
	4	3.4	4.7	4.8	3.9	3.4
5	1	2.3	4.1	2.0	2.8	2.7
	4	3.6	4.7	4.7	4.3	3.7
mean	**4**	**4.3**	**4.7**	**4.4**	**4.0**	**3.5**

Table 3. Log Reduction of PHMB Formulation in PET; 6 months, 40 °C

Trial No.	t (hr)	Sa	Pa	Sm	Ca	Fs
1	1	4.7	3.9	2.2	2.8	1.8
	4	4.7	4.7	4.6	3.4	2.8
2	1	4.7	2.8	1.8	2.9	0.8
	4	4.8	4.9	4.5	3.1	1.7
3	1	3.9	2.8	3.1	3.1	---
	4	4.9	4.8	4.6	3.6	2.6
4	1	3.2	4.4	3.3	2.9	1.0
	4	4.7	4.8	4.7	3.8	2.1
5	1	3.5	4.8	3.7	3.0	1.3
	4	4.9	4.8	4.7	4.2	2.8
mean	**4**	**4.8**	**4.8**	**4.6**	**3.6**	**2.4**

Table 4. Log Reduction of PHMB Formulation in HDPE; Initial Time

Trial No.	t (hr)	Sa	Pa	Sm	Ca	Fs
6	1	3.5	2.4	2.2	2.3	1.2
	4	4.8	4.5	4.0	3.6	2.4
7	1	4.5	2.3	2.6	2.4	1.4
	4	4.8	4.0	4.6	3.0	2.2
8	1	3.1	2.5	2.0	2.6	1.9
	4	4.7	4.7	3.4	3.2	3.0
mean	**4**	**4.8**	**4.4**	**4.0**	**3.6**	**3.9**

Table 5. Log Reduction of PHMB Formulation in HDPE; 3 Months, 40 °C

Trial No.	t (hr)	Sa	Pa	Sm	Ca	Fs
6	1	1.9	2.0	1.9	2.4	0.9
	4	4.3	3.4	4.0	3.1	2.0
7	1	3.4	2.3	3.2	2.6	0.8
	4	4.7	3.2	4.5	3.0	1.2
8	1	2.9	2.4	2.2	2.5	0.7
	4	4.6	3.1	4.0	3.1	1.3
mean	**4**	**4.5**	**3.2**	**4.2**	**3.1**	**1.5**

Table 6. Log Reduction of PHMB Formulation in HDPE; 6 months, 40 °C

Trial No.	t (hr)	Sa	Pa	Sm	Ca	Fs
6	1	1.7	2.7	2.8	1.6	0.4
	4	3.1	4.5	4.1	2.2	0.5
7	1	2.0	2.4	3.3	2.1	0.5
	4	2.9	4.7	4.7	3.2	1.1
8	1	0.9	3.3	1.4	0.9	0.1
	4	1.4	4.6	3.3	1.4	0.3
mean	**4**	**2.5**	**4.6**	**4.7**	**2.3**	**0.6**

Table 7. Summary Mean Log Reduction at 4 hours in PET

time	Sa	Pa	Sm	Ca	Fs
to	4.6	4.5	4.3	3.6	3.5
3 months 40 °C	4.3	4.7	4.4	4.0	3.5
6 months 40 °C	4.8	4.8	4.6	3.6	2.4

Table 8. Summary Mean Log Reduction at 4 hours in HDPE

time	Sa	Pa	Sm	Ca	Fs
to	4.8	4.4	4.0	3.6	3.9
3 months 40 °C	4.5	3.2	4.2	3.1	1.5
6 months 40 °C	2.5	4.6	4.7	2.3	0.6

P03346

BAUSCH & LOMB INCORPOR

TO: Law Department – Patent Group

FROM: Irene Quenville

RECORD OF INVENTION

Descriptive Title: Stability Improvement of PHMB and Alexidine based formulations in PET Bottles

Description of Invention:

In the development of ReNu-02, a next generation lens care product, the formulation (NRC03) exhibited poor chemical stability and disinfection efficacy in high density polyethylene (HDPE) bottles. HDPE bottle resins contain numerous additives, such as antioxidants, plasticizers, flame retardants, etc., that have the ability to "bloom" to the surface of the bottle and interact with formulation ingredients. This "blooming" phenomenon is typically exacerbated by the presence of surfactants, such as Tetronics and Pluronics. These resin additives are believed to interact with poly(hexamethylene biguanide) (PHMB), the disinfecting agent in NRC03, thereby rendering the disinfectant less active.

In searching for ways to improve the stability of NRC03, a change to the bottle resin was considered. Product stability was evaluated in clear bottles produced with poly(ethylene terephalate) (PET) resin. Unexpectedly, significant improvements in chemical stability and disinfection efficacy were observed in PET bottles for NRC03 stored at accelerated temperature (40°C) conditions. These accelerated temperature conditions are used to predict product shelf-life. A comparison of the PHMB stability profile in HDPE vs. PET bottles is shown in Exhibit I. A comparison of biocidal efficacy in HDPE vs. PET bottles for *Fusarium solani* is shown in Exhibit II.

Due to the poor stability exhibited by NRC03 in HDPE bottles, a second formulation (NRC07) was selected and evaluated for stability in HDPE and PET bottles. The disinfecting agent in NRC07 is Alexidine. No significant differences in the chemical stability of Alexidine were observed between HDPE and PET bottles under accelerated temperature conditions (Exhibit III). However, significantly greater biocidal efficacy was observed for *Candida albicans* in PET bottles (Exhibit IV).

1. Inventor (print full legal name) Irene Quenville — Huris No. 114674
   Home Address 74 Regan Drive, Oswego, NY 13126 — Citizenship: U.S.
   Signature/date Irene Quenville
   Witness/date

2. Inventor (print full legal name) _____ — Huris No. _____
   Home Address _____ — Citizenship: _____
   Signature/date _____ ___/___/___
   Witness/date _____ ___/___/___

EXHIBIT I

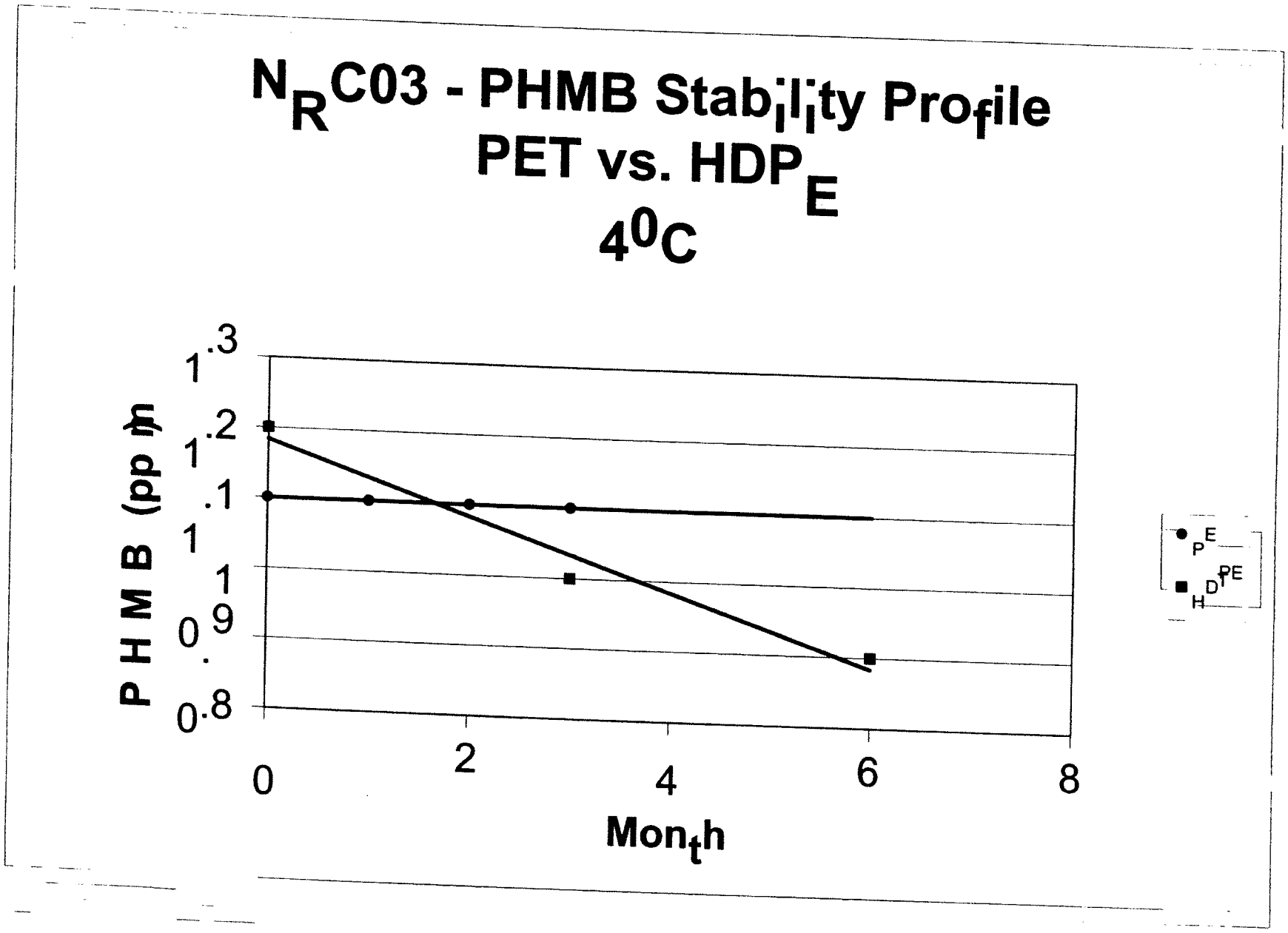
NRC03 - PHMB Stability Profile
PET vs. HDPE
40C
PHMB (ppm)
1.3
1.2
1.1
1
0.9
0.8
0
2
4
6
8
Month
PE
HDPE

EXHIBIT II

NRC03 Biocidal Efficacy

Fusarium solani